

ability of a protein. Of two regions which fit the CRAC criteria from a leukotoxin LtxA produced by a pathogenic bacterium, only one is responsible for the cholesterol binding of the toxin. Molecular dynamics simulation of peptides corresponding to both regions reveals that, despite the peptides sharing similar structural characteristics in a solution environment, this comparison is lost near a cholesterol-bearing bilayer. Near such a bilayer, the cholesterol-binding sequence shows a significant loss of secondary structure upon association with the membrane. Furthermore, these results were not observed near a pure phospholipid bilayer, indicating that this behavior is specific to cholesterol-containing membranes.

2802-Pos Board B232

Cholesterol Accessibility Sensing by Perfringolysin O Derivatives is Linked to Changes in the Size of the Oligomer

Benjamin B. Johnson¹, Robert J.C. Gilbert², Alejandro P. Heuck¹.

¹Biochemistry and Molecular Biology, University of Massachusetts, Amherst, MA, USA, ²Division of Structural Biology, Wellcome Trust Centre for Human Genetics, University of Oxford, Oxford, United Kingdom.

Perfringolysin O (PFO) is a cytolysin secreted by *Clostridium perfringens* that requires cholesterol in the target cell membrane for binding. Binding of PFO to membranes is modulated by cholesterol accessibility. No PFO binding occurs until the accessibility of cholesterol at the membrane surface reaches a certain threshold. Amino acid modifications at the tip of the PFO C-terminal domain (or domain 4) changes the threshold for how much cholesterol is required to trigger binding. These PFO derivatives are excellent candidates to develop probes to image cholesterol in cells and to study cholesterol accessibility on different cellular membranes.

The molecular mechanism for how cholesterol regulates PFO binding remains elusive. We have explored the effect of domain 4 modifications in the ability of the toxin to form arcs and rings on membranes using electron microscopy. Our data revealed that the amount of cholesterol in the membrane required to trigger PFO binding correlates with changes in the size and shape of the formed PFO oligomers.

2803-Pos Board B233

Free Energies for Trans-Membrane Pore Formation in the Presence of Arginine-Rich Peptides from Molecular Dynamics Simulations

Neha Awasthi, Jochen S. Hub.

Structural Molecular Biology, Georg-August University, Goettingen, Germany.

Antimicrobial peptides are unique and diverse group of molecules, where the amino acid composition, cationic charge and size enables them to attach and insert into plasma membrane to form pores. These trans-membrane pores eventually lead to the cell death of the microbe. Several mechanisms were proposed for the activity of antimicrobial peptides, but a quantitative understanding of those mechanisms has remained elusive. Therefore, we modeled the formation of a trans-membrane pore in the presence of a cationic Arginine-rich model peptide using coarse-grained and atomistic molecular dynamics (MD) simulations. Free energies for trans-membrane pore formation were calculated as a function of peptide concentration and lipid composition. We find that electrostatics and presence of counter-ions; esp. for the case of charged lipids and peptides, play a critical role in free energy calculations. We address the following questions: a) does insertion of a peptide or several of them, influence the free energy of pore-formation; and b) is pore-formation a required step, or is the increased disorder of the lipids sufficient to insert a peptide? Finally, we present a quantitative comparison between the free energy of trans-membrane pore in the absence and presence of N-Arginine peptides, with the aim to provide a quantitative description of the activity of antimicrobial peptides on lipid membranes.

2804-Pos Board B234

Response of GWALP Transmembrane Peptides to Incorporation of Buried Histidine Residues

Ashley N. Martfeld, Denise V. Greathouse, Roger E. Koeppe.

Chemistry and Biochemistry, University of Arkansas, Fayetteville, AR, USA.

To investigate histidine residue replacements in lipid bilayer membranes, we have employed GWALP23 (acetyl-GGALW⁵LALALALALALW¹⁹LAGA-amide) as a favorable host peptide framework. We inserted His residues into position 12 and/or 13 of GWALP23 (replacing either L12 or A13) and incorporated specific ²H-Ala labels within the helical core sequence. Solid-state ²H NMR spectra of GWALP23-H12 reveal a marked difference in peptide behavior between acidic and neutral pH conditions. At neutral pH, GWALP23-H12 and GWALP23-H13 exhibit well-defined tilted transmembrane orientations in both DOPC and DLPC bilayer membranes. Under acidic conditions GWALP23-H12 and GWALP23-H13 are highly dynamic and exhibit multiple

states. Indeed, the multi-state behavior of GWALP23-H12 and GWALP23-H13 between pH 1.5 and pH 3 resembles closely that of GWALP23-R12 at neutral pH (J. Am. Chem. Soc. 132, 5803). The dramatic change in the behavior of each peptide suggests a pK_a value of less than 3 to yield the neutral His imidazole side chain when buried in a lipid bilayer. Chemical exchange of the C2 imidazole proton for deuterium introduces a probe which potentially allows for direct observation of the His ring by solid-state ²H NMR over a range of conditions. Multiple His residues further alter the peptide properties, as GWALP23-H12,13 appears to aggregate in DLPC and DOPC bilayers over a range of pH conditions. Similar patterns are observed with GWALP23-H12,14; yet the ²H quadrupolar splittings for the $\beta = 90^\circ$ and $\beta = 0^\circ$ membrane orientations suggest different helix dynamics. Further aspects of the pH dependence of transmembrane helices having one or two histidine residues are under investigation.

2805-Pos Board B235

Influence of Cholesterol on Single Arginine-Containing Transmembrane Helical Peptides

Jordana K. Thibado, Ashley N. Martfeld, Denise V. Greathouse, Roger E. Koeppe.

Chemistry and Biochemistry, University of Arkansas, Fayetteville, AR, USA.

Polar amino acids within the helical core of a transmembrane sequence, although sparse, are essential to the structure and function of many membrane proteins. In order to examine polar residues in lipid bilayer membranes, it is useful to employ a peptide framework such as GWALP23 (acetyl-GGALWLA LALALALALALWLAGA-amide). GWALP23 and related model peptides fold into helices and adopt defined tilted orientations that can be observed in bilayer membranes by means of solid-state NMR spectra from particular labels. For example, it is convenient to include specific ²H-labeled alanine residues within the helical core of the peptide. GWALP23 is a favorable host peptide for single residue replacements due to the limited dynamic averaging of NMR observables such as the deuterium quadrupolar splittings of the alanine side chains. The goal of this project is to study the influence of arginine residues and pH on helix behavior in cholesterol-containing bilayers. GWALP23-R14 was incorporated into DOPC bilayers with varying amounts of cholesterol (0-20%). Although 10% cholesterol has little effect on the orientation of GWALP23-R14 in DOPC bilayers, solid-state ²H NMR spectra reveal a marked difference in peptide behavior when 20% cholesterol is present in the bilayers. Multiple peaks in the ²H NMR spectra of GWALP23-R14 (Ala d₄: 11^{50%} and 13^{100%}) in DOPC bilayers with 20% cholesterol at high pH suggest a multi-state behavior of the peptide. Changes in the magnitudes of the ²H quadrupolar splittings furthermore suggest that 20% cholesterol may alter the helix tilt even at lower pH. The results reveal a sensitivity of peptide helix properties to cholesterol.

2806-Pos Board B236

Influence of a Potentially Destabilizing Central Tryptophan on Transmembrane Helix Domains

Vasu Suresh Kumar, Ashley N. Martfeld, Denise V. Greathouse, Roger E. Koeppe.

Chemistry and Biochemistry, University of Arkansas, Fayetteville, AR, USA.

Synthetic model peptides, such as GWALP23 (acetyl-GGALW⁵LALALALALALW¹⁹LAGA-amide), provide a favorable "host" framework for investigations of the influence of chosen "guest" amino acids. For example, it is of interest to know the consequences of having a third, centrally located, tryptophan (Trp) within the hydrophobic core of a well characterized, anchored, transmembrane helix. It is crucial to note that the orientation and rotation of GWALP23 are sensitive to single-residue replacements, in part because the membrane-spanning helix exhibits only limited dynamic averaging of solid-state NMR observables such as the ²H quadrupolar splitting (Biophys. J. 101, 2939). A Trp residue was introduced in the 12th or 13th position of GWALP23, and specific deuterated alanine labels (²H-Ala) were included as probes within the core helical sequence. The ²H quadrupolar splittings from solid-state NMR spectra of GWALP23-W12 and GWALP23-W13 show that the peptide remains helical and retains a dominant preferred tilted transmembrane orientation (similar to GWALP23) in lipid bilayer membranes of DOPC, DLPC, and DMPC. Modified Gaussian and semi-static treatments of the dynamics yield similar conclusions. While a central Trp at position 12 or 13 does not alter the characteristics of bilayer-spanning GWALP23, incorporation of the peptide helix into the bilayer membrane becomes more difficult. The properties of W^{4, 5} GWALP23 are also being investigated, for comparison with the highly dynamic Y^{4, 5} and the less dynamic F^{4, 5} peptides. Deuterium labels at Ala3 and Ala21 will allow assessment of possible fraying of the ends of selected helices in differing lipid membrane environments.